1777-Pos Board B547
The Effect of Blebbistatin and Cytochalasin D on Force Generation in DRG Lamellipodia
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We have used optical tweezers to analyse the effect of Blebbistatin and Cytochalasin D on the force exerted by lamellipodia from developing growth cones of isolated Dorsal Root Ganglia neurons. In the presence of 20 μM Blebbistatin, a well known inhibitor of Myosin II, force generation still occurs but with a slower rate and a reduced velocity of the lamellipodium leading edge. Frequency of elementary jumps underlying force generation was reduced by approximately 50%. The distribution of amplitudes of elementary jumps was fitted with the exponential distribution, $A_+ e^{-(j+/j+*)}$ and $A_- e^{-(j-/j-*)}$, with the values of 3.4 ± 0.9 and 3.2 ± 0.8 nm for the mean size of positive j^+* and negative j^-* jumps in the presence of Blebbistatin and 5.2 ± 1.3 and 4.9 ± 1.2 nm in control conditions.
The addition of 12.5-50 nM Cytochalasin D, an inhibitor of F-actin polymerization slowed down lamellipodia motion but did not abolish force generation. In the presence of 50 nM Cytochalasin D, growth cones moved very slowly, with a velocity two times less than in control conditions. Small concentrations of both drugs significantly decreased the occurrence of shovel like events, during which the lamellipodium had waves of protrusion/retraction and lifted up by 1-3 microns. Therefore, shovel like events could be mediated by specific molecular mechanisms, different from those underlying lateral protrusions and retractions.

1778-Pos Board B548
The Effect of Jasplakinolide and Cyclodextrin on Force Generation in DRG Lamellipodia
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We have used optical tweezers to analyse the effect of Jasplakinolide and Cyclodextrin on the force exerted by lamellipodia from developing growth cones of isolated Dorsal Root Ganglia (DRG) neurons. 25 nM Jasplakinolide, known to reduce actin filament turnover, reduced both the maximal exerted force and maximal velocity with which the lamellipodium leading edge protrudes. 2.5 mM Cyclodextrin, known to reduce membrane rigidity by removing cholesterol from the membrane, had the opposite effect: lamellipodia treated with Cyclodextrin exerted a larger force and their leading edge could advance with a larger velocity. Neither Jasplakinolide nor Cyclodextrin affected force and velocity during lamellipodia retraction. Amplitude and frequency of elementary jumps underlying force generation were reduced by Jasplakinolide, while Cyclodextrin increased their size without affecting their frequency. These results indicate that membrane rigidity and actin turnover modulate force generation during protrusion but not in a significant way during retraction.

Cardiac Muscle II

1779-Pos Board B549
Dissociation Between Force and Calcium after a Step Change in Frequency
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The force frequency response (FFR) is one of the major physiological regulators which govern alterations in contractile function in the body in which an increase in frequency of pacing leads to an increase in force production and a decrease in rate of contractile kinetics. Typically studies on FFR have been conducted at steady state where the changes in contractile function have been elicited by changes in myofilament phosphorylation. Here we aim to determine the role of alterations in calcium homeostasis and myofilament sensitization during the transition from one steady state of contractile function to another.
Trabeculae, excised from the right ventricular free wall of New Zealand white rabbits were loaded with Rhod-AM calcium indicator dye and stimulated to contract from 1 to 4 Hz. Data was analyzed through customized *Labview* software.
We hypothesize that the transition from one steady state to another occurs in two phases; the rapid/early phase is due to increases in calcium transient amplitude and diastolic calcium levels, and the gradual/latent phase is due to alterations in phosphorylation state of myofilament proteins such as troponin I (TnI) and myosin light chain 2 (MLC2). During our experiments we are able to measure calcium and developed force simultaneously. Despite previous studies which show alterations in myofilament calcium sensitivity occurring at steady state, we are able to show that during the immediate phase calcium and force change with each other, while at the latent phase there is an increase in diastolic calcium level that is not paralleled by the diastolic force. This suggests a myofilament desensitization during this latent phase that is not seen in the early transitory period as originally thought.

1780-Pos Board B550
Symmetric Modulation of Cross-Bridge Kinetics by Sarcomere Velocity during Shortening and Lengthening in Cardiac Trabeculae; A New Insight on Sarcomere Dynamics
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There is a controversy whether cross-bridge (XB) dynamics is determined by XB displacement, filaments velocity or the load experienced by each XB. The study tests these three hypotheses at the sarcomere level. **Methods:** Trabeculae were isolated from rat right ventricles (n=9). Sarcomere length was measured by laser diffraction. Changes in the number of strong XBs (N_{XB}) were evaluated by measuring the dynamic stiffness. Ramp stretches and releases at different velocities and onset times were imposed over isometric sarcomere contractions. **Results:** Stretches yielded parallel increase in the force and stiffness, at all stretch velocities. The force per XB during stretch was constant, independent of the velocity, and equal to the isometric force per XB. This observation is incongruent with a load dependent kinetics. The instantaneous stiffness during the ramp stretches and releases was normalized by the isometric stiffness. Identical changes in the normalized stiffness were observed when identical ramp perturbations (stretch or release) were imposed at different onset times during the twitches. Thus changes in the normalized N_{XB} are not dominated by XB recruitment processes, although the number of available XBs varies with time during the twitch. The normalized stiffness development rate linearly depended on the lengthening velocity with a slope of 6.73 ± 0.98 [1/μm]. During shortening the normalize stiffness decline rate depended linearly on the shortening velocity with identical slope of 6.70 ± 1.43 [1/μm]. **Conclusions:** The symmetrical dependence of the normalized stiffness development rate on the velocity and the independence on the perturbation onset time are conveniently explained by the velocity dependent hypothesis in the framework of an integrated sarcomere, where there is a cooperative interaction between the XBs. This mechanism explains the force-velocity relationship and the muscle high contractile efficiency.

1781-Pos Board B551
Genetic Variation Results in Differing Age-Related Drosophila Myocardial Stiffness
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Rapid aging of Drosophila melanogaster makes it suitable for studying age-related mechanical changes such as impaired heart function. The Drosophila heart consists of a cardiomyocyte tube bonded to a thin ventral muscle layer. It has previously been observed that there is a decrease in the diastolic heart tube diameter with age (>20%) in the control fly strain yellow-white (yw), which we hypothesized was due to an increase in passive tension due to myocardial stiffening. We sought to investigate this through nanoindentation in both yw and other control lines. Using a modified Hertzian analysis method, we probed the stiffness of juvenile and geriatric female yw flies and found age-related stiffening, a finding consistent with myocyte stiffening in other cardiac systems over time. Stiffness at the cardiac cell-cell junctions was 1.8 ± 0.1 vs. 3.8 ± 0.3 kPa in 1 week old flies which increased to 3.8 ± 0.3 kPa in 5 week flies. In contrast, white (w1118) flies were found to have no age-related stiffening. Consistently, w1118 also have a less significant decrease in diastolic diameter with age (<10%). Moreover, our analysis method is

capable of detecting mechanical separation of the muscle layers, which was found to occur more frequently in w1118 than yw (0.41 ± 0.04 vs. 0.00 ± 0.02 μm separation, respectively, 1 week flies). Detection of mechanical separation between muscle layers via nanoindentation was modeled and verified in a microfabricated polydimethylsiloxane system. This first in situ mechanical analysis of a living myocardium revealed differences in cardiac mechanics due to age and suggest that aspects of the mechanical properties of the aging phenotype differ between Drosophila strains. We investigation on other laboratory Drosophila wildtype strains to assess the impact of diverse genetic backgrounds or mutations on age-related myocardial stiffening and cardiomyopathy.

1782-Pos Board B552

In Situ Mechanical Analysis of Genetic Modification and Aging on Soft, Bilayered Drosophila Myocardium

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Drosophila melanogaster is a genetically malleable organism with a short life span, making it a tractable system in which to study mechanical effects of genetic perturbation and aging on tissues, such as impaired heart function. However, Drosophila heart tube studies can be hampered by its bilayered structure: a ventral muscle layer covers the contractile cardiomyocytes. We have refined an atomic force microscopy-based analysis to measure individual mechanical components of soft composite materials. The technique was verified using bilayered polydimethylsiloxane. Its biological utility was further demonstrated by its ability to resolve stiffness changes due to cardiac-specific RNA interference to reduce cardiomyocyte myofibrillar assembly or due to aging in Drosophila myocardial layers. Female yellow-white (yw) flies experience decreased diastolic diameter with age (>20%) while cardiomyocytes stiffened more than two-fold with age (1.8 ± 0.1 vs. 3.8 ± 0.3 kPa in 1 and 5 week old flies, respectively) at cell-cell junctions. Cardiac-specific RNA-interference against myosin heavy chain severely impaired contraction and reduced stiffness after 1 week (1.0 ± 0.1 vs. 1.8 ± 0.1 kPa) without altering ventral muscle stiffness. This method provides a platform to assess the mechanics of soft biological composite systems and for the first time permits direct measurement of how genetic perturbations, aging, and disease can impact cardiac function in situ.

1783-Pos Board B553

Exploration and Suppression of Cardiac Amyloidosis Induced by Huntington's Disease-Causing Amyloid in the Drosophila Heart Model

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Accumulation of amyloids is associated with cardiomyopathy; however, the precise mechanism that leads to defective heart structure and function is unknown. Amyloid-like inclusions have been detected in patients with Huntington's disease (HD), which is caused by an expanded polyglutamine (Poly-Q) repeat in the Huntington (HTT) protein. HD patients also demonstrate a greater occurrence of cardiovascular events, presumably as a result of toxic amyloid accumulation due to global protein misfolding and/or oxidative stress. To explore cardiac defects associated with HD-causing amyloid protein, we used the UAS-Gal4 system and a cardiac-specific driver (Hand-Gal4) to express mutant HTT with short (UAS-*Httex1*-PolyQ25) and disease-causing expanded (UAS-*Httex1*-PolyQ72) Poly-Q in the *Drosophila* heart. Expression of disease causing Poly-Q in 1 and 3 week old fly hearts resulted in severe cardiac defects as evidenced by prolonged diastolic and systolic intervals, a significantly increased incidence of arrhythmias and extreme cardiac dilation that was accompanied by a significant decrease in cardiac contractility (reduced fractional shortening). Structural analysis showed myocardial cells with noticeably reduced myofibrillar content, myofibrillar disorganization and the presence of amyloid-aggregates. No such physiological and structural defects were seen upon expression of short Poly-Q under similar conditions. To take advantage of our genetic model and to further explore the mechanism underlying the PolyQ-induced cardiac defects, we co-expressed expanded Poly-Q with either the antioxidant enzyme superoxide dismutase (SOD) or a chaperone protein UNC-45. Our preliminary results suggest that cardiac dilation is reduced and cardiac performance is enhanced upon co-expression of SOD or UNC-45. Thus we have developed a novel *Drosophila* model that allows us to explore cardiac defects associated with the accumulation of HD-causing amyloid and to elucidate the mechanisms underlying cardiac failure in HD patients.

1784-Pos Board B554

Multi-Scale Modeling of Patient-Specific Ventricular Geometry, Fiber Structure, and Biomechanics

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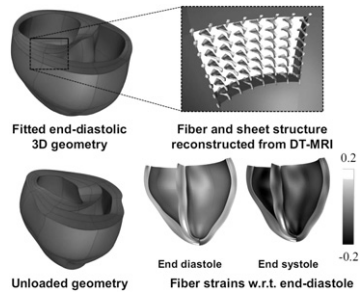
Patient-specific image data of the heart can now be obtained through advanced medical imaging. This data combined with clinical measurements can potentially be integrated into patient-specific computational models of regional cardiac function. We have developed a pipeline for patient-specific ventricular biomechanics simulations in the failing heart. Three-dimensional ventricular geometry was segmented from CT or MRI data at end-diastole from patients with congestive heart failure. Human myofiber and sheet architecture was estimated using eigenvectors computed from Diffusion Tensor MRI obtained in an isolated, fixed human organ donor heart and mapped to the patient-specific geometric model using large-deformation diffeomorphic mapping. Passive myocardial properties were optimized using semi-automated methods while simultaneously computing the unloaded reference geometry. Active cardiac-muscle contraction properties were optimized to match ventricular pressures measured by cardiac catheterization. Finally, echocardiographic data and an adaptation algorithm (CircAdapt) were used to estimate parameters of a lumped-parameter closed-loop model of the circulation. These methods were validated in three heart failure patients who gave informed consent at the San Diego VA Medical Center by comparing simulation results with echocardiographic measurements of regional wall motion and with predictions of empirical formulas derived from previous clinical studies.



1785-Pos Board B555

Cross-Bridge Cycling Kinetics in Intact Multicellular Cardiac Muscle at Physiological Temperature: Impact of Muscle Length

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The contractility of the heart is physiologically determined by load, frequency and β-adrenergic stimulation. It has been shown that these regulatory mechanisms involve post-translational modifications of myofilament proteins that can potentially influence the rate of cross-bridge cycling, an important determinant of cardiac output. We set out to develop a method for measuring cross-bridge cycling rate in intact cardiac muscle preparations where the cascades of post-translational signaling are functionally intact. With the use of a K^+ contracture protocol, we were able to induce a steady-state tension in intact trabeculae and measure the rate of tension redevelopment (k_{tr}), an index for cross-bridge cycling rate. We utilized this technique in order to investigate the effect of load on cross-bridge cycling rate. In cardiac trabeculae isolated from Brown Norway rats (n=11), the rate of tension redevelopment was measured twice at L_{opt} (optimal length) and at L_{90} (corresponding to 90% of optimal length) in each muscle. The k_{tr} for the L_{90} was 45.1 ± 7.6 s^{-1} and it was significantly decreased to 27.7 ± 3.3 s^{-1} as the muscles were stretched to their L_{opt} ($P < 0.05$). The k_{tr} for each length was measured a second time in order to show the reproducibility of the system. There was no significant difference between the duplicate measurements of each length ($P = 0.84$). In addition, we were able to apply these experiments in mammals that more closely reflect the human situation (such as the rabbit and dog) and muscle preparations isolated from explanted human hearts. This technique permits the studying of cross-bridge cycling kinetics in intact muscles in a reproducible and reliable manner, where the impact of signaling cascades leading to post-translational modifications can be studied.

1786-Pos Board B556

Differential Twitch Kinetics in Engineered Cardiac Tissue Expressing Human Cardiac Myosins

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Congestive heart failure is a debilitating disease in which the principal pathology is impaired ventricular contractility leading to diminished cardiac output, and previous work indicates that reduced contractility is based in part on the